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Chapter 4

Role of Tumour Markers in Diagnosis and Follow up of Colorectal Cancer — Potential for Future Research

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Immunodiagnostics of Colorectal cancer — CEA and CA 19-9

Colorectal cancer is the second most common cancer in terms of incidence in men and women. Another concern is the high rate of morbidity and mortality in patients with this cancer. Therefore, researchers are constantly searching for new diagnostic methods that would enable the early detection of recurrent, clinically asymptomatic periods. The development of clinical immunodiagnostics has enriched oncology with the possibility of determining the quantity of glycoproteins and glycolipids in the blood of patients with cancer. These are called neoplastic markers. The usefulness of a neoplastic marker assay has been confirmed in diagnosing alimentary tract neoplasms, mainly in the early post-operative detection of a recurrence of neoplastic disease and in the evaluation of the efficacy of surgery.

According to an account published by The European Group on Tumor Markers (EGTM) of 2003, CEA is the main marker that is used in detecting colorectal cancer. It is important to point out, however, that approximately 10-15 % of patients do not produce CEA at all or that it is secreted in only minimal amounts. In such cases, the normal level of CEA concentration does not exclude the existence of a neoplasm even at an advanced stage. Therefore, the use of CA 19-9 as a tumor marker in diagnostics has been proposed.

1.1.1. Characteristic of CEA tumour marker

Carcinoembryonic antigen is a glycoprotein that contains about 60% carbohydrates. CEAs have epitopes that are specific to the neoplasm and epitopes that connect antibodies against nonspecific cross-reacting antigens (NCA, NCA2, BGP). Its upper normal range is 3 ng/ml [1, 2]

1.1.2. Characteristic of CA 19-9 tumour marker

The CA 19-9 Antigen is associated with gastrointestinal cancers. It occurs in the sialyated Lewis A blood group antigen that is produced in a small amount in the salivary and bronchial glands as well as in the pancreatic and bile ducts. This marker is very useful in the diagnosis of gastrointestinal cancers such as gastric, pancreatic, bile duct cancers and pancreatitis. Its upper normal range is 37 U/ml, but in approximately 1% of healthy people, concentrations reaching 120 U/ml have been detected [1, 2].

1.2. Aim of the study

The purpose of the study was to estimate the usefulness of selected neoplastic markers – conditioned by their location in the pre-operative and post-operative histological evaluations of patients with gastrointestinal cancers.

1.3. Material and methods

256 patients, both won men and women, aged 19-86, in whom colorectal cancer was diagnosed and histopathology was confirmed, were included into the research that was performed between 1991-1998.

Patients were divided into two groups according to the progression of the disease on the TMN scale and patients with a proctologic neoplasm on the Dukes and TMN scales. Neoplasm markers were marked in serum using commercial kits (blood samples were collected from the cubital vein and stored at -20°C after centrifugation). CEA and CA 19-9 were detected using the MEIA method using an Abbott's kit (USA). The upper normal range in healthy subjects is 3 ng/ml for CEA and 37 U/ml for CA 19-9.

The detection of neoplastic markers was performed in the Independent Laboratory of Clinical Immunodiagnostics at State Hospital No. 5 in Sosnowiec, Poland. Blood samples were collected preoperatively, in the first, second and third months after surgery and next after every 3 months for 2-5 years.

1.4. Results

The detection of neoplastic markers was extended about lab tests, abdominal ultrasonography; CT was performed in certain cases. Results were worked out using the t-Student test, the Cochran-Cox test, variance analysis (ANOVA) and the Shapiro-Wilk test for hardly large test.

Table I describes the results of the division of patients according to the stage of the disease on the TNM scale. The pre-operative CEA and CA 19-9 concentrations is presented in Figure 1 and Figure 2.

A pre-operative elevation of the CEA concentration in serum was found in 182 patients (71%). CEA did not exceed the normal range in the Dukes A group. CA 19-9 was increased in 83 (32%) patients in the Dukes C and D groups. The mean concentration of CEA and CA 19-9 changed

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according to the stage of the disease and were: Dukes A group – CEA ($\bar{x}$=1.82 ng/ml, CA 19-9 $\bar{x}$=12.45 U/ml, Dukes B group – CEA $\bar{x}$=5.97 ng/ml, CA 19-9 $\bar{x}$=15.37 U/ml, Dukes C group – CEA $\bar{x}$=7.42 ng/ml, CA 19-9 $\bar{x}$=55.73 U/ml, Dukes D group – CEA $\bar{x}$=17.97 ng/ml, CA 19-9 70.42 U/ml. In the post-operative follow-up, in which the Dukes D group was excluded, a recurrence was found in 53 patients, an elevation of CEA was found in 47 patients (88.6%) and CA 19-9 was found in 36 patients (67.9%). The recurrence was detected in 100% of the patients when an elevation of CEA CA 19-9 was accepted as a criterion. The results are shown in Figure 3.

Numbers of patients	Dukes scale	TNM scale
8	A	3 – T1N0M0 5 – T2N0M0
61	B	61 – T3N0M0
94	C	37 – T3N1M0 57 – T3N2M0
7	D	2 – T4N2M1 5 – T4N2M1

Table 1. The stages of the disease on the Dukes and TNM scales.

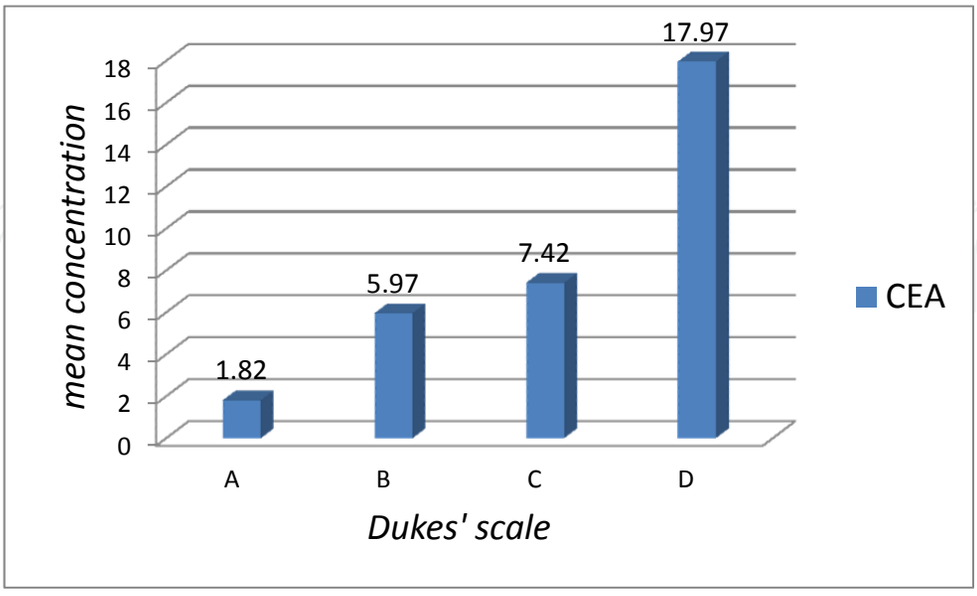

Figure 1. Mean concentration of CEA markers in pre-operative patients.

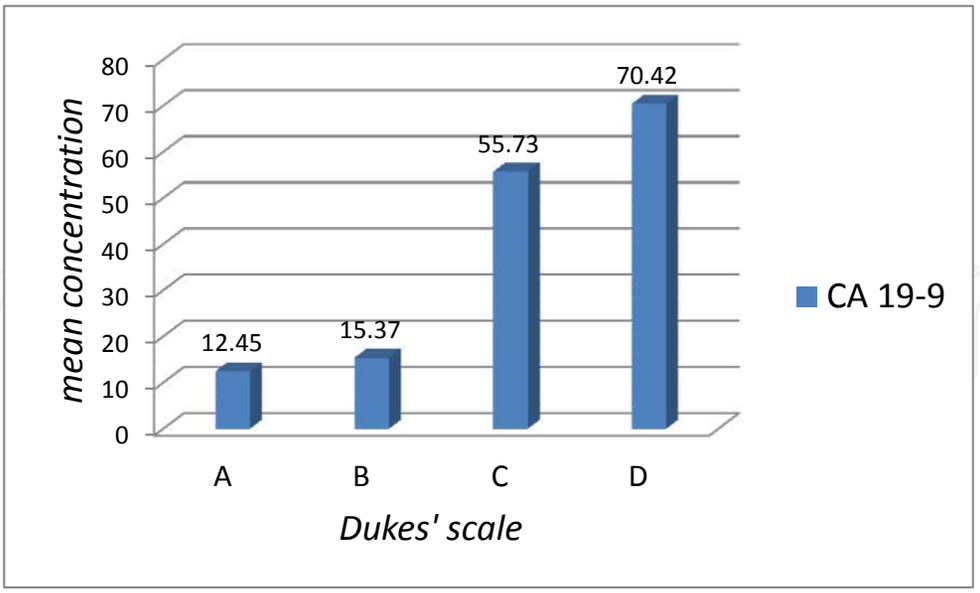

Figure 2. Mean concentration of CA 19-9 markers in pre-operative patients.

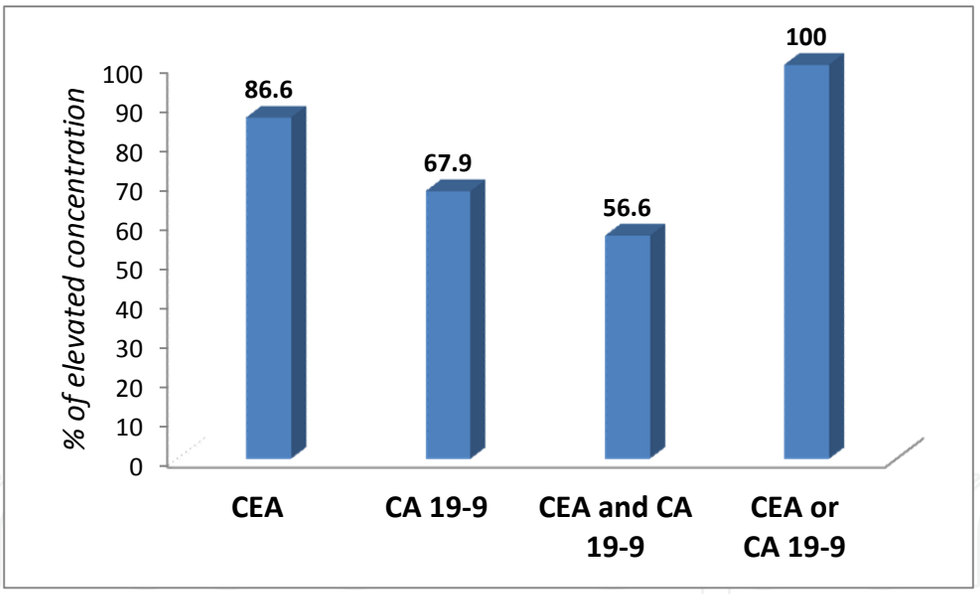

Figure 3. Percentage of CEA and CA 19-9 concentration in patients with a recurrence.

1.5. Discussion

Although the dreams of Bates et al. [3] to find an ideal marker for an active neoplastic process, i.e. that they have a different effect depending on the location of an organ and are absent in healthy people, were frustrated, neoplastic markers are now widely used in clinical diagnostics, usually for patients who have undergone surgery to remove cancerous tissue. Studies that lasted several years revealed that in order to estimate the efficacy of surgery, to detect a

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recurrence of a neoplastic process in the asymptomatic phase and to estimate the effectiveness of supplementary therapy, a determination of markers in the serum of patients plays a crucial role [1, 2, 4, 5, 6, 7, 8, 9].

Gold and Freedman [10] isolated carcinoembryonic antigen in colon cancer in 1966. They thought that it was specific to colorectal adenocarcinomas. The process of a quantitative determination of CEA in systemic fluids was described shortly thereafter, which indicated that more cancers produce CEA than had been previously thought. Moreover, it was found that its serum concentration may be higher than the normal range in non-neoplastic diseases such as pneumonia, bronchitis, tuberculosis, infections of the urinary tract and also in 30% of smokers [1, 10].

CEA is increased in non-neoplastic diseases of the intestines like colitis ulcerosa and Crohn's disease [1, 4, 11]. This information appeared to reduce the clinical value of a CEA assay; however, the development of monoclonal antibodies against CEA improved the specificity of the assays. This antigen is not present in the serum of all patients, even in a case of a recurrence, which was shown in studies that lasted for several years. Therefore, it is important to enhance clinical immunodiagnostics through the use of other markers (epitopes), which can use the information provided by the assay of CEA. The studies included 256 patients divided according to the stage of the cancer on the Dukes and TNM scales. Two neoplastic cancers CEA and CA 19-9 were determined in all 256 patients. Increased CEA was found in 182 patients (71%) and CA 19-9 was found in 83 patients (32%).

An analysis of the results revealed that in addition to CEA, CA 19-9 is an especially helpful marker. This agrees with the reports of Dienst et al. [12], who found increased concentrations of CEA in 49-58.5% of patients and increased concentrations of CA 19-9 in 21-67% of patients. However, Filela et al. [5] observed increased concentrations of CEA in 61% of patients and increased concentrations of CA 19-9 in 35% of patients. The concentration of both markers changed depending on the stage of the disease. CEA and CA 19-9 concentrations were within normal limits in the Dukes A group; the mean concentration of CEA was above the normal limits, 5.97 ng/ml, and CA 19-9 was within the normal limits in the Dukes B group. In the Dukes C group, the mean concentration of CEA was 7.42 ng/ml and the mean concentration of CA 19-9 was 55.73 U/ml. Similar results can be found in literature. Szymendera [2], Nowacki [7] and Lindmark et al. [13] revealed that in the advanced stages of colon cancer, a percentage of patients have elevated CEA and CEA concentrations. However, about 10-15% of patients do not secrete CEA.

The literature reveals that about 11-13% of patients with histopathologically confirmed colorectal cancer do not "produce" CEA and that an assay of these markers can lead to false negative results [2, 4, 9]. In these cases, the presence of advanced cancer is not excluded by a CEA concentration within the normal limits. CA 19-9 is the marker of first choice in this group of patients. The addition of CA 19-9 to an assay of CEA increased the sensitivity from 71% to 83.6% in our studies; however, 12.5% of patients with CEA within the normal limits had elevated CA 19-9. A positive correlation of CEA, CA 19-9 and the Dukes scale was revealed. Similar results were obtained by other authors. Fillela et al. [5] revealed that multifactoral analysis indicates the prognostic significance of CA 19-9 independent of the Dukes scale. New

information about the CA 19-9 antigen has been revealed in recent years. CA 19-9 is sialofucololactotetraosyl and is included in the group of E-selectines. E-selectines enable a rise of remote metastases that is caused by the adhesion of neoplastic cells to epithelial cells in macrocirculation vessels. A pre-operative statistical analysis showed that the probability of recurrence is higher in cases where there is a higher CEA concentration before a treatment. However, Filela et al. [5] revealed that the risk of recurrence is 2.95 times greater in pre-operative patients with an increased concentration of CA 19-9 than in patients with a normal concentration of CA 19-9. 170 of 256 the patients who underwent surgery were tested in the follow-up phase. Patients in the Dukes D group were not tested. A recurrence was observed in 53 of the 170 patients (31%). The mean concentration of CEA was 20.71 ng/ml in the Dukes B group and 20.55 ng/ml in the Dukes C group. The mean concentration of CA 19-9 was 61.61 U/ml and 197.18 U/ml, respectively. A recurrence was detected in 100% of the patients when an increased concentration of CEA or CA 19-9 was used as a criterion. A recurrence was detected in 88.6% of patients when only CEA was estimated and 67.9% of patients when only CA 19-9 was estimated. The differentiation of a local neoplasm and remote metastases is difficult. Szymendera [2] reported that a concentration of CEA that is greater than 20 indicates metastases in the liver, while a small concentration of CEA or CA 19-9 might indicate metastases in the bones or lymph nodes.

1.6. Conclusions

1. Simultaneous detection of CEA and CA 19-9 should be the first immunodiagnostic test in patients suspected of having colorectal cancer.
2. The use of carcinoembryonic antigen is advisable in order to monitor the course of the disease in the case of an increased serum concentration of CEA and CA 19-9.
3. An increased concentration of CA 19-9 along with a normal lack of CEA in the serum of patients with colorectal adenocarcinomas is unfavorable prognostically.

The continuous development of immunodiagnostic methods and the production of monoclonal antibodies can bring new neoplastic markers into diagnostics. One of these is the TPS (Tissue Polipeptide Specific Antigen). Its structure is similar to the TPA (Tissue Polipeptide Antigen). Reports in the last 2-3 years suggest the great value of the determination of TPS in the serum of patients, including patients with gastrointestinal cancers, especially for the early detection of release and estimation of therapy effectiveness. TPS is a marker of cell proliferation and an increase in its concentration in serum often precedes the markers of a tumor

2. Scientific literature indicates interest of a cellular proliferation marker — TPS

A review of medical reports from recent years shows an increasing interest in estimating TPS levels mainly in oncologic diagnostics. Estimating TPS- concentration (which is a marker connected with the proliferation of neoplastic cells) is very important for monitoring patients

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who have undergone cancer surgery (esp. of the digestive tract, but also for breast and ovarian cancer) proceeding the clinical symptoms of metastasis for 2-7 months.

2.1. Characteristic of tissue polypeptide specific antigen (TPS) — Soluble fragments of cytokeratine 18

TPS (tissue polypeptide specific antigen) is a new marker of cellular proliferation. The antibody directed against TPS enables the determination of the concentration of the soluble fragments of cytokeratin 18 [14]. TPS was introduced into oncological immunodiagnostics by Bjorklund. It has one of the two active epitopes of TPA (tissue polypeptide antigen) that are detectable by the monoclonal antibody M3. TPS is a singular conjugated polypeptide chain that is created in the S and G2 phases of the cellular cycle and is released immediately after mitosis. It has 33 antigen determiners, two of which are connected with the activity of a tumor. TPS is strictly connected with the proliferation of neoplastic cells and is a function of the velocity of cell divisions [15].

2.2. Clinical results of serum concentration of TPS in patients with colorectal cancer

2.2.1. Aim of the study

1. To estimate pre-operative CEA and TPS concentrations in the blood serum of patients with colorectal cancer and rectal carcinomas depending on the advancement of their disease.
2. To attempt to determine whether TPS provides additional information that cannot be obtained from CEA tests only.

2.2.2. Material and methods

178 patients (101 men and 77 women) aged 22-86 years who had been diagnosed with colorectal cancer and had undergone surgery in the years 1991-2002 were included in the study. The patients were being treated at the Department of General Surgery and Coloproctology of the Medical University of Silesia in Sosnowiec. The CEA concentration was determined in the patients' blood serum using the MEIA method and commercial sets from Abbott (USA). TPS was determined using the enzyme-immunological method (EIA) and sets from BEKI (Sweden). The normal concentration of CEA was determined as 3 ng/ml and in the case of TPS – 90 U/l.

The criteria for choosing patients for the research: 178 patients whose pre-operative diagnostics confirmed the existence of a colon or rectal adenocarcinoma in a histopathological examination.

The criteria for excluding patients from the research. The research excluded patients who were diagnosed with:

- an inflammation of the large intestine (colitis ulcerosa, Leśniowski-Crohn disease),
- chronic kidney diseases,

- chronic liver diseases,
- an inflammation of the rheumatoid joints,
- autoimmunological diseases caused by autoimmunity (Hashimoto, Graves-Basedov, thyroid cysts),
- diabetes, or
- chronic infections.

The largest number of patients in the research was in the Dukes C group – 89 patients (50%). The fewest number of patients was in the Dukes A group – 8 patients (10.11%)(Table II)

Numbers of patients	Dukes scale	TNM scale
8	A	3 – T1N0M0 5 – T2N0M0
62	B	62 – T3N0M0
89	C	53 – T3N1M0 36 – T3N2M0
19	D	11 – T4N2M1 8 – T4N2M1

Table 2. The degree of the clinical advancement of colon and rectal carcinomas according to Dukes.

2.2.2.1. Statistical methods

All results were statistically measured using the Statistica 6.0 program from StatSoft Inc.

2.2.3. Results

No increased abnormal CEA concentration was found in any patient in the Dukes A subgroup. An increased amount of CEA was found in 37 cases (59.7%) in the Dukes B subgroup, in 75 patients (83.9%) in the Dukes C subgroup and in 17 cases (89.57%) in the Dukes D subgroup.

Another profile was observed when determining TPS. An increased concentration was found in 3 patients (37.5%) in the Dukes A subgroup. An increased concentration was found in 48 cases (77.4%) in the Dukes B subgroup, in 59 cases (65.5%) in the Dukes C subgroup and in 6 cases (31.6%) in the Dukes D subgroup. (Table III)

Dukes	CEA [%]	TPS [%]
A	0,00	37,5
B	59,68	77,41935
C	83,91	65,51724
D	89,47	31,57895

Table 3. The percentage of patients with an increased abnormal concentration of CEA and TPS in relation to the Dukes scale.

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In cases where the division according to the degree of advancement in the whole group of 178 patients was not taken into account, the sensitivity for pre-operative CEA concentration was 72.5% and for TPS – 65.2%. When only cases with increased levels of CEA and TPS concentrations were taken into account, the sensitivity of the test increased to 82.6%.

The concentration of CEA was: 2.34 ng/ml in the Dukes A subgroup, 5.71 ng/ml in the Dukes B subgroup, 8.66 ng/ml in the Dukes C subgroup and 19.97 ng/ml in the Dukes D subgroup, respectively. (Table IV)

Dukes	Number of patients	Average amount	Standard deviation	Standard error	-95% CI	+95% CI
A	8	2,34	0,362284	0,128087	2,03462	2,64038
B	62	5,71	4,385849	0,557003	4,59636	6,82396
C	89	8,66	7,035047	0,745713	7,17906	10,14296
D	19	19,97	9,826148	2,254273	15,23553	24,70763

Table 4. CEA concentration in patients before surgery in relation to the degree of the advancement of the cancer according to Dukes.

Another characteristic was observed in the case of the determination of TPS concentration. The highest average pre-operative TPS concentration was found in the Dukes C subgroup – 226.7 U/l. It was 107.4 U/l in the Dukes A subgroup and 181.2 U/l in the Dukes B subgroup. However, the average amount measured in the Dukes D subgroup was 167.37 U/l, which may be connected with a decrease in proliferation. (Table V)

Dukes	Number of patients	Average amount	Standard deviation	Standard error	-95% CI	+95% CI
A	8	107,41	60,0221	21,22102	57,2328	157,5922
B	62	181,20	75,9138	9,64106	161,9283	200,4853
C	89	226,71	126,6201	13,42170	200,0392	253,3848
D	19	167,37	145,3267	33,34024	97,3295	237,4200

Table 5. TPS concentration in patients before operation in relation to the degree of the advancement of the cancer according to Dukes.

A recurrence of the disease was detected in 47 patients (Dukes B and C). When the concentration of CEA was used, recurrence was detected in 89.4% of patients and when the concentration of TPS was used, recurrence was detected in 80.85% of patients. If the criterion was an elevated

concentration of CEA or TPS, recurrence was detected in of 100% patients. The concentration of CEA in patients with a recurrence was $\bar{x}$=12.82 ± 4.73 ng/ml in the Dukes B group and $\bar{x}$=13.5 ± 7.69 ng/ml in the Dukes C group. The concentration of TPS was $\bar{x}$=282.95 ± 56.08 U/l in the Dukes B group and C $\bar{x}$= 313.77 ± 116.62 U/l in the Dukes C group.

2.2.4. Discussion

In diagnosing carcinomas of the digestive system, particularly in the case of colon and rectal carcinomas, the carcinoembryonic antigen (CEA) still remains the "gold standard" [16, 17, 18]. However, great expectations are connected with the introduction of the soluble fragments of cytokeratin 18 (TPS) into the immunodiagnostics of colorectal cancer because TPS reflects the velocity of cell divisions [19].

Our results of pre-operative CEA concentrations are similar to those that have been reported by other researchers. Treska et al. obtained the highest sensitivity of CEA assessment from 45% to 80% depending on the degree of the progression of cancer [20]. Similar results were reported by Turoldo et al. and Marchena et al. [21, 22].

The main aim of the presented research was to estimate the usefulness of determining TPS concentration. The TPS sensitivity was 65.17% in our own clinical research. The highest pre-operative sensitivity equaling 70% was reported by Plebani et al. [23].

We also observed that the sensitivity of the test increased when the results of the determination of TPS and CEA were combined. An abnormal pre-operative CEA concentration was recorded in 129 patients (72.47%). When determining TPS concentration, 116 patients were found to have increased abnormal levels (65.17%). When the established criterion was an increased level of TPS or CEA, then the sensitivity of the test increased to 82.31%. Lindmark et al. using the CEA, CA 19-9, CA 50 and TPS tests proved their correlation with one another; however, only the TPS concentration test had the highest diagnostic sensitivity [18].

It is important to stress that adding TPS determination to the standard tests used for detecting and monitoring colon and rectal carcinomas has recently been approved by the European Group of Tumor Markers. According to the EGTM tests, adding TPS determination to the list of "mass tumor" markers enables an increase in sensitivity, particularly in the earlier stages of colorectal cancer [24].

2.2.5. Conclusions

1. The profile of the activity of pre-operative TPS concentration in the blood serum of patients with colorectal cancer in relation to the degree of the advancement of the cancer is different from that observed for CEA.
2. Determination of TPS concentration in patients with colorectal cancer provides essential information necessary to confirm the cancer, particularly at the earliest stages of its advancement.

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3. Apoptosis and proliferation – Bcl-2

The mechanism of malignancy is considered to be an imbalance between apoptosis and the processes of proliferation. A phenotype that is resistant to apoptosis is one of the major features of cancer cells. Recently, attention has been drawn to the function of a number of proteins that inhibit the process of apoptosis within a tumor. To date, only a few works connected with the assessment of apoptosis proteins serum concentrations have been published. Most of the works about apoptosis concern immunohistochemistry studies.

This study attempts to find an answer to the question of whether the serum concentration of antiapoptotic the Bcl-2 protein provides additional information for the post-operative monitoring of patients with colorectal cancer.

3.1. Material and methods

The research was conducted on 46 patients (21 with a B Astler-Coller's stage cancer and 25 with a C Astler-Coller's stage cancer) was it colon cancer, who underwent surgery (resection RO). Their ages ranged from 47 to 85 (average age 67); sex (19 women, 27 men). The patients were divided into 2 groups: I –patients with a recurrence of cancer and II – patients without a recurrence of cancer. The control group consisted of 20 healthy people, mainly medical staff. The average CEA concentration in this group was 1.6 ng/ml+/- 0.43; TPS: 48.67U/l+/- 9.1; Bcl2: 0.31ng/ml+/- 0.13. The period of the observation of the patients and conducting the research was 1-5 years. The recurrence of the disease or the lack of a recurrence was confirmed using a physical examination and additional examinations during the oncological follow-up. Ten ml of venous shunt blood was collected from each patient. The serum was frozen at -20°C after centrifuging-. The blood for testing was collected one day before the surgery and 1, 3, 6 and 12 months after the surgery. CEA was measured using MEIA method and a commercial set from ABBOT (USA). The standard concentration for a healthy person was adopted as 3ng/ml. TPS was measured using the EIA method and sets from Beki Diagnostic Bromma (Sweden). The standard for a healthy person does not exceed 90 U/I. Bcl-2 concentration was labeled using the ELISA method using SORIN-BIOMEDICA tests (Italy). A standard for healthy people is 0.5 ng/ml. The results obtained were analyzed statistically. Calculations were done using Microsoft Excel 2003. The Ethical Committee at the Silesian Medical University approved the studies.

3.2. Results

Of the 46 patients who underwent surgery, a recurrence was detected in 14 patients including 6 with an initial stage of a tumor – B according to the Dukes classification as modified by Astler-Coller and 8 – degree C. The detection time of the recurrence was from 6 to 23 months. Most of the recurrences were distant metastases: 9 in the liver and 2 in the lungs. A local recurrence was observed in the intestinal stapling or retroperitoneal space in 3 patients. (Table VI) and (Figures 4, 5 and 6)

It was established that the Bcl-2 concentration was statistically significantly higher in the recurrence group than in the non-recurrence group when examined 1, 3, 6 and 12 months after the surgery. The TPS concentration was statistically significantly higher in the recurrence group than in the non-recurrence group when examined before and 3, 6 and 12 months after the surgery. The concentration of the antigen, i.e. CEA, was statistically significantly higher in the recurrence group in relation to the non-recurrence group, as was TPS in the pre-operative determinations and 3, 6 and 12 months after the surgery. Bcl-2, TPS and CEA serum concentrations were unrelated to the Astler-Coller stage of colorectal cancer. However, insignificantly higher concentrations of CEA at degree C than at degree B were observed. There was also no dependence related to the sex, the age of a patient, the original location of the tumor and the recurrence. Correlations between the concentrations of the determined parameters in all patients (with a recurrence and without a recurrence) were also noticed. A strong correlation between the concentrations of Bcl-2 and TPS proteins occurred 12 months after the surgery in the recurrence group.

Feature	No recurrence				Recurrence			
	Average	SD	Min.	Max.	Average	SD	Min.	Max.
Bcl2_0	8,09	6,82	0,41	24,39	10,39	6,78	0,57	23,02
Bcl2_1	7,34	6,07	1,74	28,41	8,87	3,17	4,09	14,61
Bcl2_3	7,40	6,58	0,50	29,79	12,15	8,14	0,55	30,00
Bcl2_6	6,97	6,99	0,41	26,40	17,50	7,98	1,98	29,74
Bcl2_12	8,38	7,67	1,47	25,09	20,52	7,03	12,14	29,81
CEA_0	5,26	7,22	1,70	42,60	5,75	2,60	2,30	11,90
CEA_1	2,61	1,00	1,10	6,30	2,46	0,53	1,70	3,00
CEA_3	1,87	0,90	0,40	3,70	4,94	3,88	1,20	17,40
CEA_6	2,21	2,19	0,30	11,90	10,72	5,74	3,70	20,30
CEA_12	2,57	2,97	0,40	14,60	14,86	13,92	1,30	41,00
TPS_0	98,9	19,1	60,4	160,3	118,1	31,1	60,7	168,9
TPS_1	92,8	16,8	64,6	143,7	101,6	17,7	80,3	144,1
TPS_3	95,9	27,0	64,7	188,3	125,2	31,0	90,7	193,7
TPS_6	96,8	35,7	70,3	197,6	152,0	49,0	80,4	279,1
TPS_12	96,6	37,9	70,4	207,4	152,4	34,2	100,0	190,4
Age	66,2	10,9	47,0	85,0	70,4	4,0	64,0	77,0

Table 6. Values of the basic description parameters (0 – preoperative results and 1-, 3-, 6-, and 12 months after the surgery.

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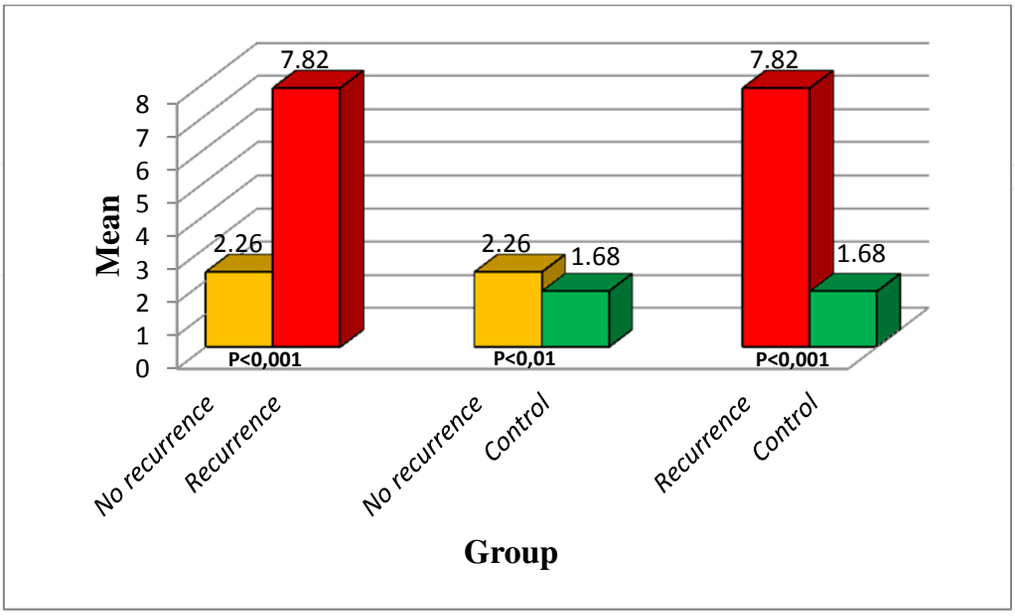

Figure 4. Results of the evaluation of post-operative CEA levels in the group without recurrence, with recurrence and the control group.

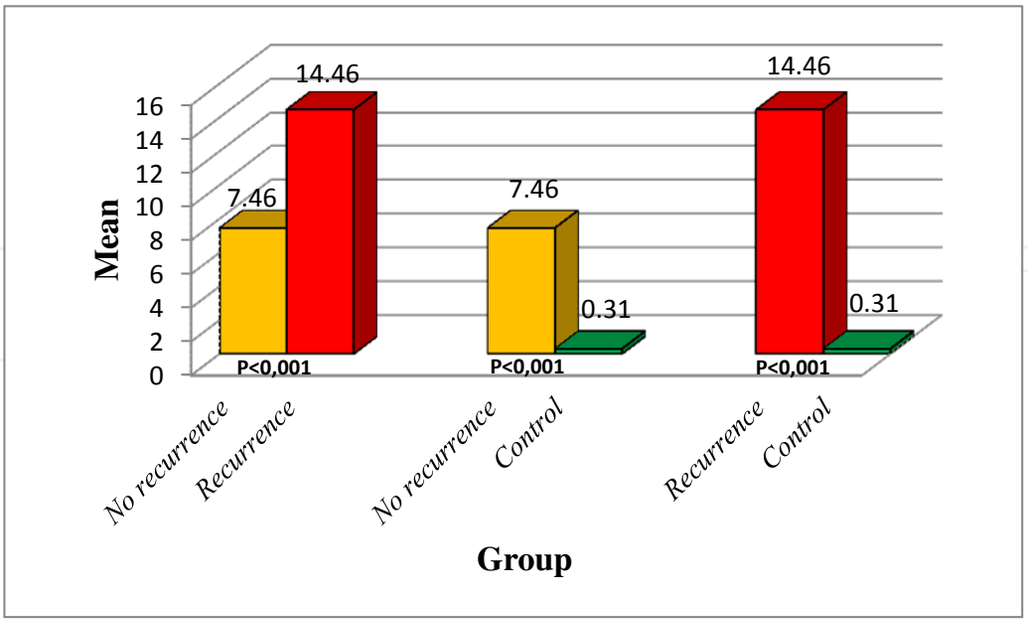

Figure 5. Results of the evaluation of post-operative Bcl-2 levels in the group without recurrence, the recurrence and the control group.

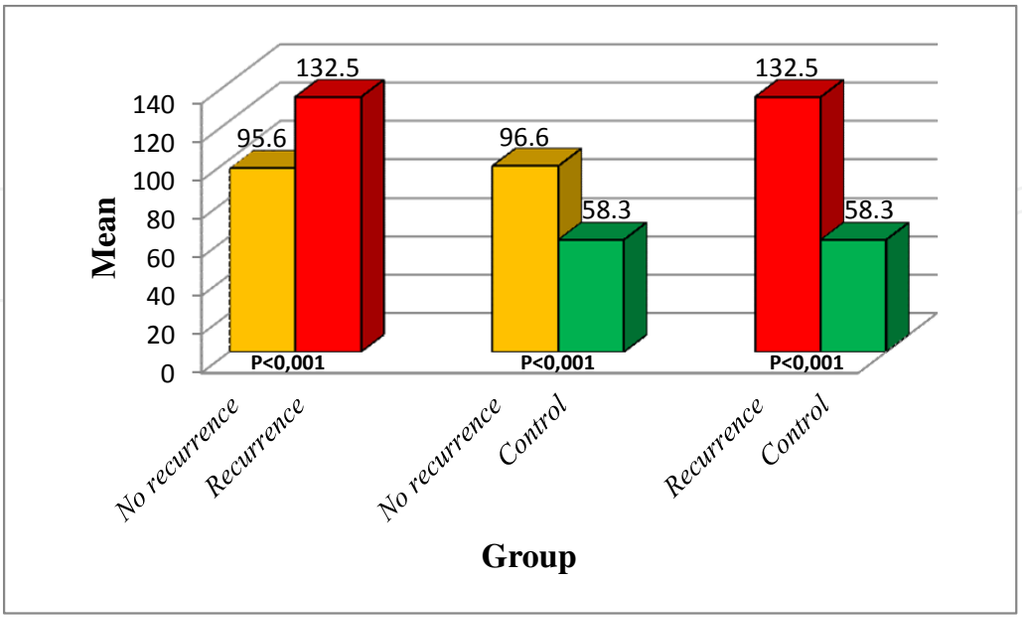

Figure 6. Results of the evaluation of post-operative TPS levels in the group without recurrence, the recurrence and the control group.

3.3. Discussion

The European Tumor Markers Association recommended CEA as a useful clinical marker in the diagnosis and monitoring of patients with colorectal cancer in 2003. To date, many scientific researchers have been shown that the addition of tumor markers in the diagnosis of patients with colorectal cancer is necessary. Colorectal cancer, like breast and lung cancer, reveals a high expression of the antiapoptotic proteins: Bcl-2, Bcl-XL, PED, Il-4, which are secreted by tumor cells, strengthens that expression and protects neoplastic cells by environmental death signals.

A low expression of BAX expression correlates with disease recurrence of the disease in preoperatively irradiated rectal carcinomas and is connected with a worse response. A decrease in the expression of BAX indicates the worst response to chemotherapy and reduces the life expectancy of patients [25, 26, 27].

Our results show that an increase of Bcl-2 in the serum of patients with colorectal cancer is bad prognostically. A high concentration ($\bar{x}$=14.46 ng/ml) was observed in the group with a recurrence of the disease. The concentration of the Bcl-2 protein has been correlated with TPS – a marker of cell proliferation. A high correlation in 12th month after surgery may confirm that the suppression of the apoptosis of cancer cells increases their proliferation.

At the present time the apoptotic process and the process of cell proliferation are the targets of many researchers in different areas of specialization.

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3.4. Conclusions

1. A statistically significant excess of Bcl-2 in patients who have a recurrence of colorectal cancer makes information saying about the suppression of cancer cells apoptosis.
2. A statistically significant increase of the concentration of TPS in the group with a recurrence seems to indicate that the suppression of apoptosis is conductive to an excessive proliferation of cancer cells.
3. The findings obtained can mean that the evaluation of Bcl-2 and TPS may be complementary to CEA determinations in the post-operative follow-up of patients with colorectal cancer.

4. Angiogenesis – VEGF

The process of angiogenesis, which is the creation of new blood vessels, plays an important role in the development and metastasis of cancer. It can be initiated by tumor cell hypoxia, tumor suppressor gene mutations and oncogenes. As a result of the accumulation of these processes, tumor cells activate the angiogenic factors. The main factor involved in angiogenesis is VEGF-A. Blocking angiogenesis is one of the ways of preventing the development and metastasis of cancer and is the future of cancer therapy.

4.1. Aim of the study

1. An assessment of the concentration of VEGF-A in the blood serum of patients with colorectal cancer.
2. An attempt to answer the question of whether the determination of VEGF-A provides clinically meaningful information in the post-operative monitoring of patients.

4.2. Material and methods

117 patients underwent surgery for colorectal cancer in the years 2004-2009. Patients were divided according to the Dukes and TNM classifications. The control group consisted of 20 healthy volunteers. A recurrence was detected in 35 patients in the period of 623 months after the surgery, including 11 patients in the Dukes B group and 24 patients in the Dukes C group. Patients with a recurrence were grouped together, while the remaining 71 patients made up the group without a recurrence.

The concentration of CEA and VEGF-A was determined in all of the patients before the surgery and 1, 3, 6 and 12 months after the surgery. CEA was determined by MEIA using Abbott kits (USA); the standard concentration in healthy people is 3 ng/ml. VEGF-A was determined by ELISA using BIOMEDICA Sorin kits (Italy); the standard concentration in healthy people is 350 pg/ml.

The results were analyzed statistically. ROC curves were marked for the diagnostic parameters studied.

4.3. Results

The pre-operative mean CEA concentrations differed significantly in all three degrees of the severity of the disease (p<0.01). In the post-operative control, patients demonstrated statistically significant differences in CEA concentrations 3, 6 and 12 months after surgery with the largest concentration at the month follow-up (p<0,001). (Figure 7).

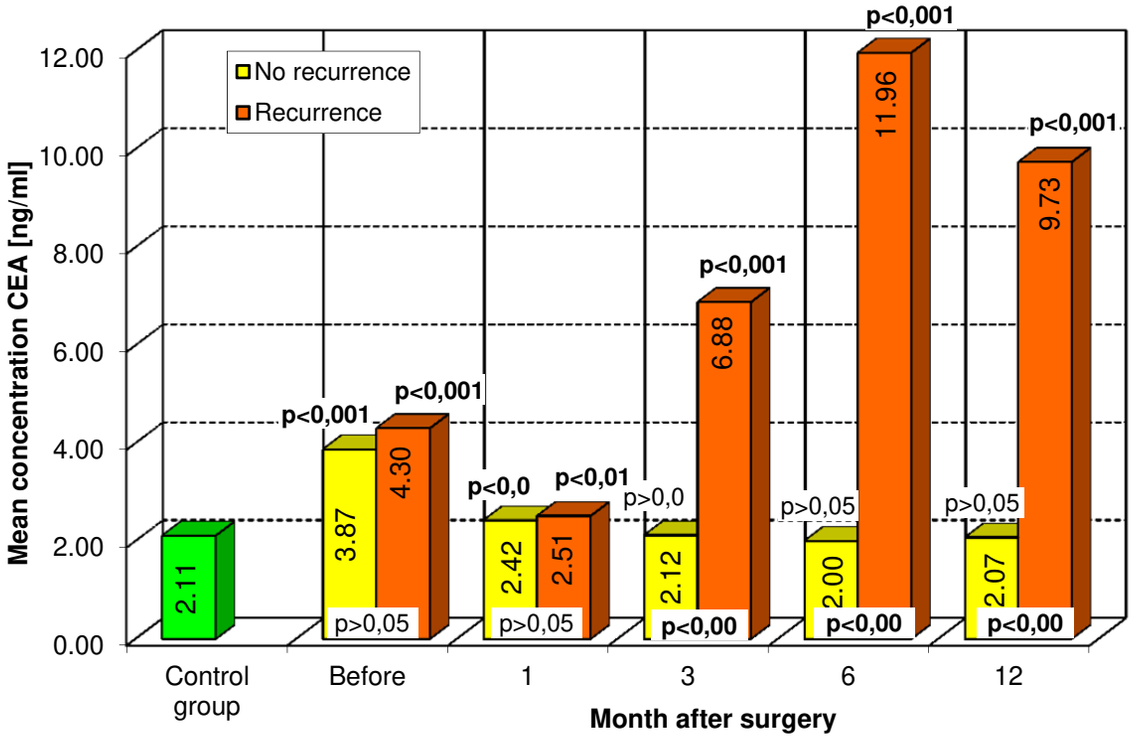

Figure 7. Evaluation of the concentration of CEA in the control group and in the groups of patients with and without a recurrence of a tumor in the subsequent stages of observation.

Most recurrences were detected during this period. The average concentrations of CEA in patients without a recurrence was 2.18 ng/ml and in patients with a recurrence 7.58 ng/ml. The ROC curves analysis showed a concentration of CEA of 3.1 ng/ml as early as 3 months after the surgery, which confirms the recurrence of the cancer (Figure 8).

Pre-operatively, a high concentration of VEGF-A was found in each stage of the disease; however, it showed no difference in levels of statistical significance. Throughout the period of post-operative observation, patients demonstrated a very high statistical significance between the group without a recurrence and those with a recurrence of the neoplastic process (p<0,001). (Figure 9)

The average concentration in patients without a recurrence was 294.24 pg/ml, while in patients with a recurrence it was 501.89 pg/ml. ROC curves showed the usefulness of VEGF-A in detecting a recurrence a month after surgery and a concentration of 412 pg/ml, it is confirmed (Figure 10).

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In addition, a high, statistically significant correlation between VEGF-A and CEA was demonstrated 3, 6 and 12 months after surgery (Figure 11 and 12).

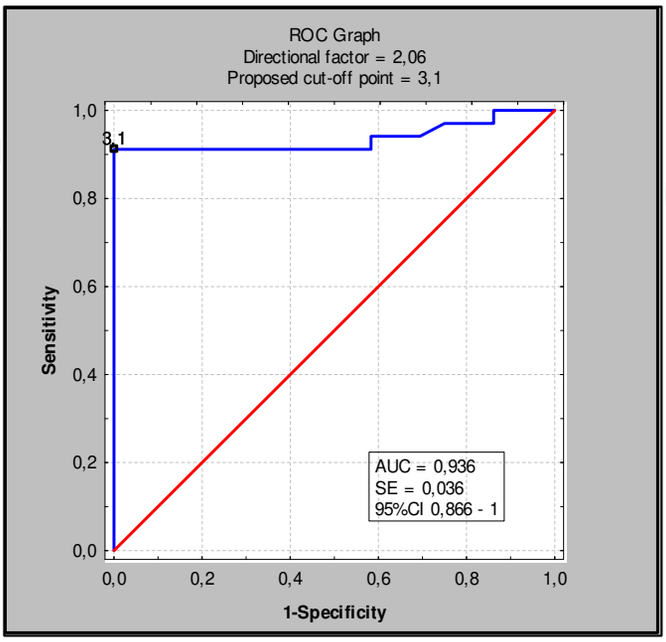

Figure 8. ROC curve for the concentration of CEA determined at the 3-month follow-up.

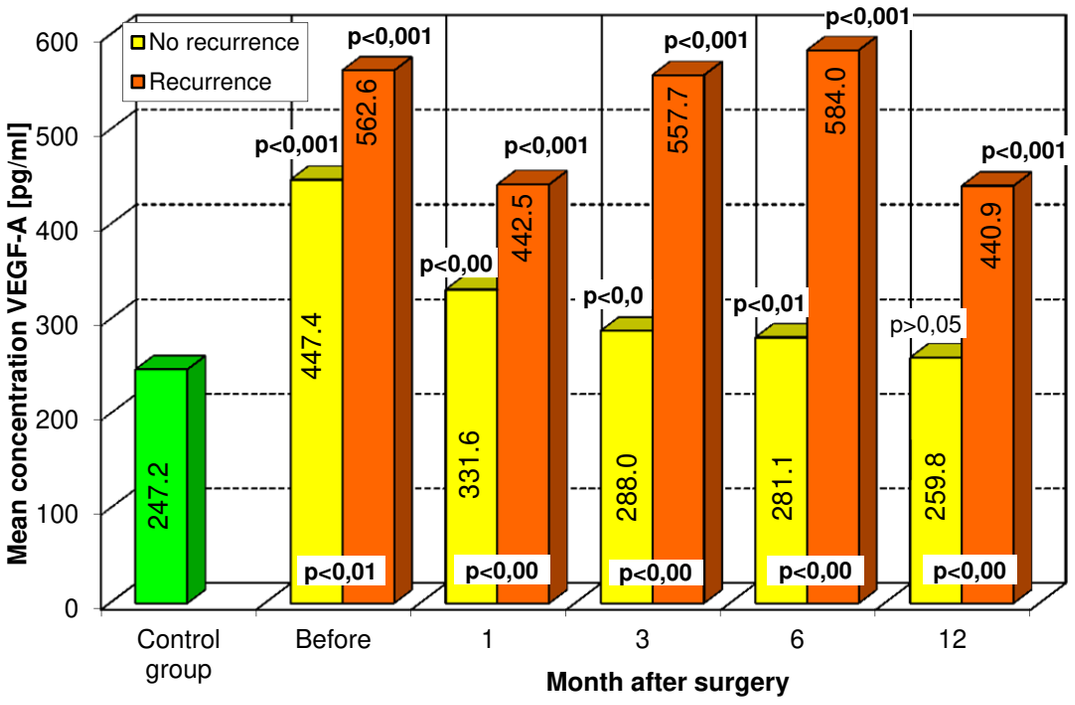

Figure 9. Evaluation of the concentration of VEGF-A in the control group and in the group of patients with and without a recurrence of a tumor in the subsequent stages of observation.

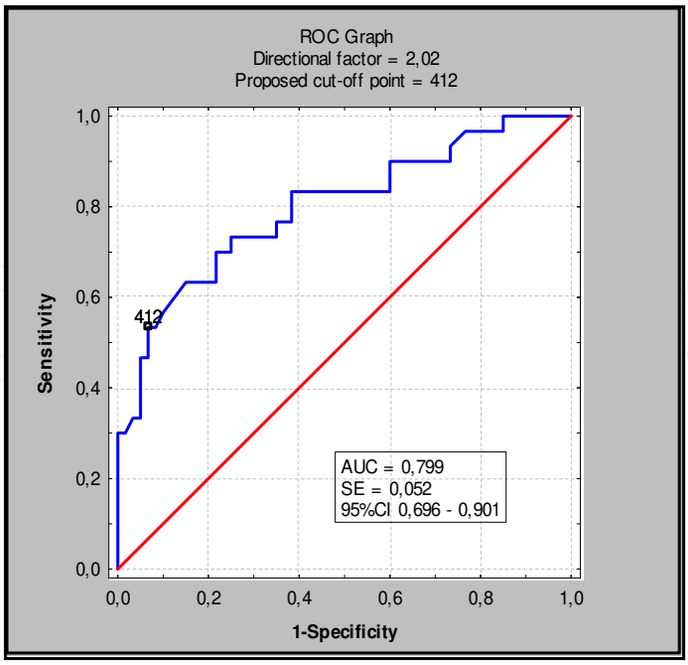

Figure 10. ROC curve for the concentration of CEA determined at a one-month follow-up.

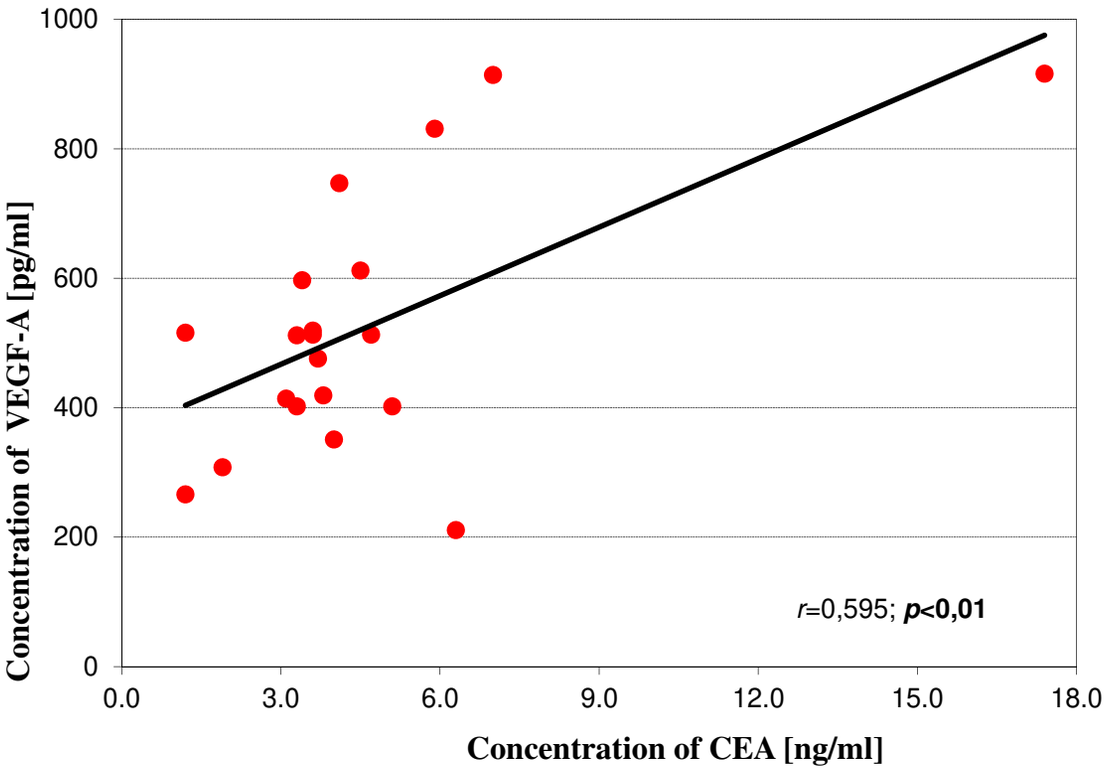

Figure 11. Correlation between the concentrations of CEA and VEGF-A in patients with a recurrence of a tumor 3 months after surgery.

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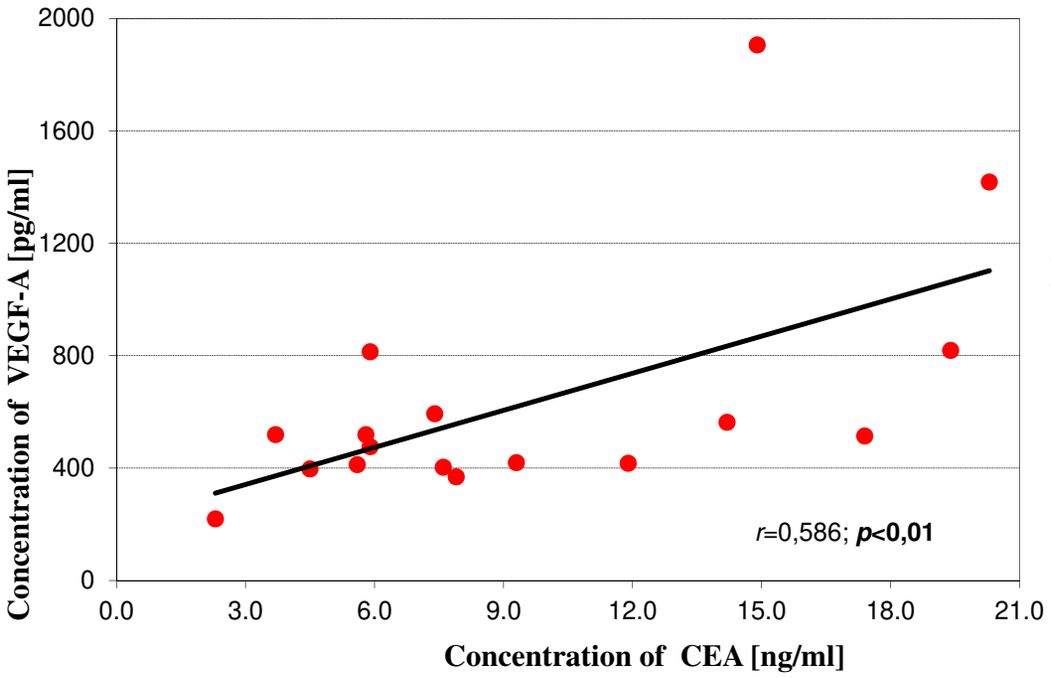

Figure 12. Correlation between the concentrations of CEA and VEGF-A in patients with a recurrence of a tumor 6 months after surgery.

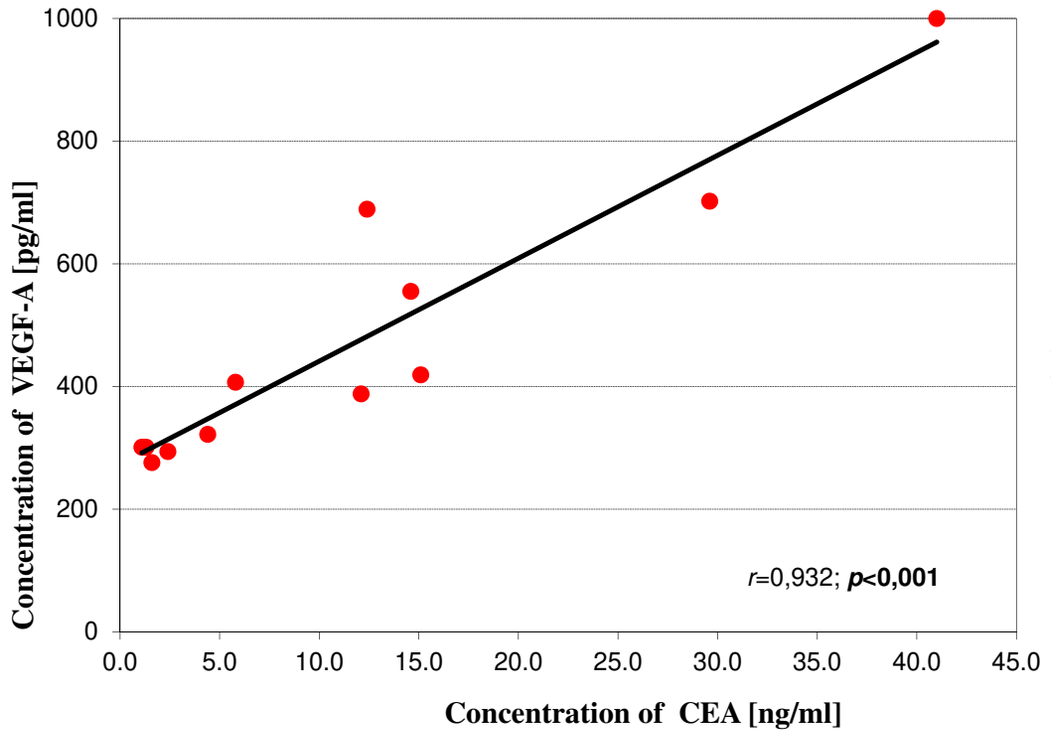

Figure 13. Correlation between the concentrations of CEA and VEGF-A in patients with a recurrence of a tumor 12 months after surgery.

4.4. Discussion

Our results are similar to the results shown by Bombardieri [28], Treska [29], Kokocińska [30] and many others. CEA was discussed in the first part of this chapter. VEGF-A was examined in the blood of patients after the surgery.

We did not find any statistically significant differences between the Dukes classification. In contrast, Fujisaki et al. [31] and Fuhrmann-Benzakein et al. [32] observed such a correlation. The highest concentration of VEGF was observed in patients with a liver metastasis.

Chung et al. [33] and Ohta et al. [34] reported that VEGF may be considered as a proliferation and prognostic factor. A high expression or concentration of VEGF indicates the possibility of the recurrence of a disease in a relatively short time.

Afify et al. [35] also indicated that the concentration of VEGF is very useful in detecting a recurrence of the disease and a metastasis to the liver.

However, Werther et al. and Karatzas et al. [36] reported that A high pre-operative concentration of VEGF suggests liver metastasis in the post-surgery period. Our results confirm those of Afify, Werther and Chung.

The results of our researches show a statistically significantly correlation between VEGF-A and CEA. It is possible that VEGF-A may stimulate the proliferation of tumor cells. To date, only Chung et al. and Ohta et al. have confirmed a connection of VEGF with the proliferation of tumor cells and with the development of cancer [33, 34].

All researches suggest that VEGF-A be added to the immunodiagnostics of CEA in patients with colorectal cancer.

5. Conclusions

1. A statistically significant increase in VEGF-A in patients with a recurrence of a tumor in the early post-operative period supports the usefulness of the inclusion of this marker for monitoring patients, especially in planning their antiangiogenic therapy.
2. The high correlation between CEA and VEGF-A seems to indicate that the concentration of VEGF-A has a close relationship with the proliferation of cells and the development of cancer.

6. Summary

A review of the scientific literature on colorectal diseases over the last 20 years indicates the continued development of Clinical Immunodiagnostics and the “gold standard”, which is CEA, but also showed the usefulness and necessity of adding new markers: TPS, Bcl-2, VEGF and their receptors.

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In the future, proteins, gene products, phenotyping of patients (determining the phenotype of patients) and molecular cytology should also be added.

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The chapter was prepared based on results of research at the Institute of Tumor Markers, which were then compared to the results obtained by other researchers.

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